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Distribution and diversity of Microfungi in Cooum River bank soils, Chennai, Tamil Nadu, India

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Abstract
The soil samples from five sites along the banks of Cooum River flowing through Chennai district of Tamil Nadu was screened for the presence of fungi of Microfungi. Soil dilution plating method was used to isolate fungi. A total of 31 species belonging to 22genera could be recovered from the soil samples studied. Soil samples of the site, Egmore showed high species diversity whereas low species diversity was recorded in soils of the site, Napier Bridge. Some of the fungal species isolated during the study are *Apergillus niger*, *Aspergillus terreus*, *Aspergills candidus*, *Penicillium* spp., *Curvularia lunata* and *Humicola griseus*. The percentage of Aspergilli was found more than 50% in three sites. Computation of Correspondence analysis revealed the presence of distinct microfungal assemblage in sampling sites.

Keywords: Cooum River, soil, Microfungi, species diversity, correspondence analysis

Introduction

Fungi are unique group of organisms, different from all other in their behaviour and cellular organization. The uniqueness of fungi is reflected in the fact that they have the status of a kingdom equivalent to the plant and animal kingdoms [1]. Fungi are ubiquitous in distribution. Recent estimate on number of fungal species indicate that there are about 12 million fungal species on earth [2]. Further, fungi represent second most species rich group after insects [3]. Fungi permeate different environments and play key roles as decomposers, mutualists and pathogens. The extreme environments are not exception for the survival of fungi. To attest this several vagarious environments have endorsed their presence which include bottom of the deadsea [4], Antarctic glaciers [5], Deserts [6], Gut of insects [7], deep oceanic sediments [8], Soils of Saltern [9], and Peat soil [10], Hypersaline waters [11].

Soil is a highly complex medium, an ecosystem with multiple abiotic and biotic components. It is not an isolated niche but consists of myriads of minute and microscopic habitats and microenvironments thus making it a multiple niches [12]. Soil is one of the natural niche for the fungi. Fungi are active in the litter and humus layers as well as in the uppermost soil layer. Fungi are most abundant in the upper 10 centimeters of soil and are chiefly limited to upper 30 centimeters [12]. Fungi are very successful inhabitants of soil, due to their high plasticity and their capacity to adopt various forms in response to adverse or unfavorable conditions [13]. Due to their ability to produce a wide variety of extracellular enzymes, they are able to break down all kinds of organic matter, decomposing soil components and thereby regulating the balance of carbon and nutrients [14]. In addition, many species of fungi possess the ability to act as an effective biosorbent of toxic metals such as cadmium, copper, mercury, lead, etc., [15]. The abiotic and biotic conditions prevailing in the soil plays crucial role in the diversity and functioning of fungi in soil ecosystem [16, 17]. Anthropogenic disturbances like cultivation, erosion and contamination etc., affected soil ecosystem especially the microbial wealth of the soil. Different soils have been screened for the presence of fungi like Forest soils [18], Antarctic soils [19], Acidic soils [20], Beach soil [21], Fresh and Marine water sediments [22, 23]. Cooum River flows through chennai, the capital of Tamil Nadu and there are studies of physiochemical parameters [24]. And the efforts focused on restoration of the Cooum river [25].The present study is aimed to document and understand the distribution and diversity of the microfungi from the soils of this river bank which has been contaminated due to anthropogenic activities.

Materials and Methods

Description of the Collection site

The River Couum originates from village called Sattari of Tiruvallur district around 65 km from Chennai [26]. It flows through Poonamallee and it enters the Chennai District at Arumbakkam. It then passes Choolaimedu, Chetpet, Egmore, Chintadripet, and Napier Bridge and finally drains into Bay of Bengal. The industrial effluents and domestic sewage from in and around the course of the river is drained into it rendering the water highly polluted.

Collection of soil sample

The soil samples were collected from Cooum river bank, Chennai. (13.0827° N, 80.2707° E). Five sites, (Chetput-CHE, Egmore-EGM, Chindadripet-CHI, Chepauk-CHP and Nepiar bridge-NAP) were selected as sampling site to document the soil microfungi (Fig.1). For each site, soil samples from approximately 4 cm below the ground surface were collected randomly from 10 regions spread approximately 1 km distance along the river bank. From each region approximately 200 gm of soil was collected. The 2 kg of soil thus collected were mixed thoroughly and brought to the laboratory in a sterile polythene bag. The soil samples were processed within 24 h of collection.

The physical and chemical parameter of the soils were tested in CVR Labs (P) Ltd., Chennai (Table 1)

Method of Sterilization and Media

Media and Glass ware other than Petridishes were sterilized in an autoclave at a pressure of 103kPa for 20min. Petridishes were sterilized in a hot air oven at 160^0C for 3h. Potato Dextrose Agar (PDA) medium amended with chloramphenicol (150mg/litre) was used for isolating fungi from soil.

Method used of isolating fungi

Soil dilution plating method [27] was used to isolate fungi from the soil samples. In this method one gram of the soil sample was dispersed thoroughly in 10ml of sterile distilled water. From this sample solution 1 ml was transferred to 9 ml of sterile water using micropipette. The resulting solution was mixed well and from this 1 ml was pipetted out into a conical flask containing 9 ml of sterile distilled water. From this dilution (10^{-3}) one ml was transferred into a sterile petridish containing antibiotic amended PDA medium. Six replicates were maintained and the data obtained in this method was used for ecological analysis.

Incubation and Identification

The petri dishes were incubated in light chamber for 7days. The light regimen was 12h light followed by 12 h darkness. The light chamber had a blank of three 4 foot Philips day light fluorescent lamps. The incubation temperature was 26 ± 1^0C. The Petri dishes were observed periodically and the fungal colonies were transferred to fresh PDA slants and were maintained at 8^0C in a refrigerator. The fungi that sporulated were identified using standard identification manuals [28, 29, 30, 31]. The fungi which did not sporulate were included as sterile forms and based on the morphology of the colony (colour, margin, texture, etc.,) they were assigned code numbers.

Analysis of Results

Ecological parameters were used for determining the fungal population of the soil sample. Number of propagules per gram of soil and were calculated by adopting the following formulae

$$\text{Number of propagules/gm of soil} = \frac{\text{Average number of colonies/plate}}{\text{Weight of the soil}} \times \text{dilution factor}$$

$$\text{Percentage Occurrence of Apergilli} = \frac{\text{Total number of all colonies of } \textit{Aspergillus} \text{ species in 6 plates}}{\text{Total no. of colonies of all the species in 6 plates}} \times 100$$

Statistical analysis

Correspondence analysis was used to compare the fungal assemblage of soils at different sites studied. Fishers' alpha was used to estimate the species diversity of the soil sample. The above analysis were calculated using statistical package Biodiversity Pro (The Natural History Museum and The Scottish association for Marine sciences) freeware application.

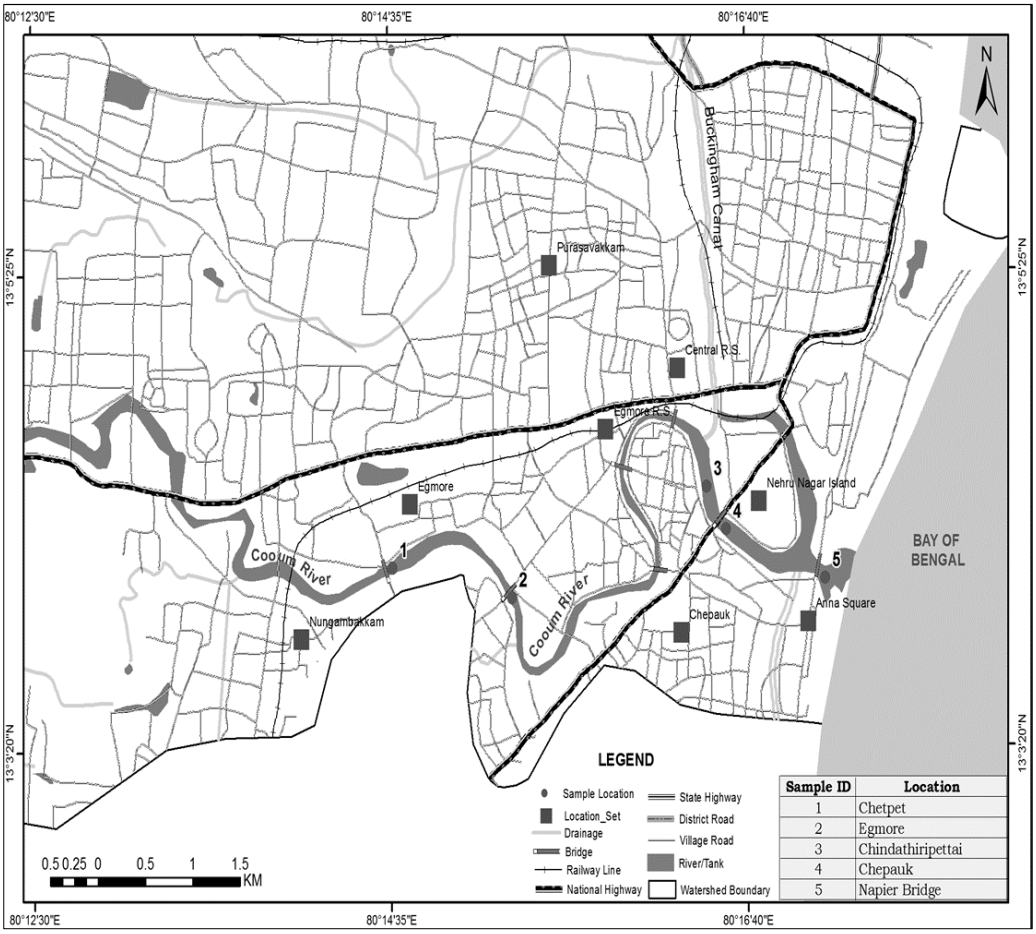


Fig 1

Table 1: Physical and Chemical parameters of the soil

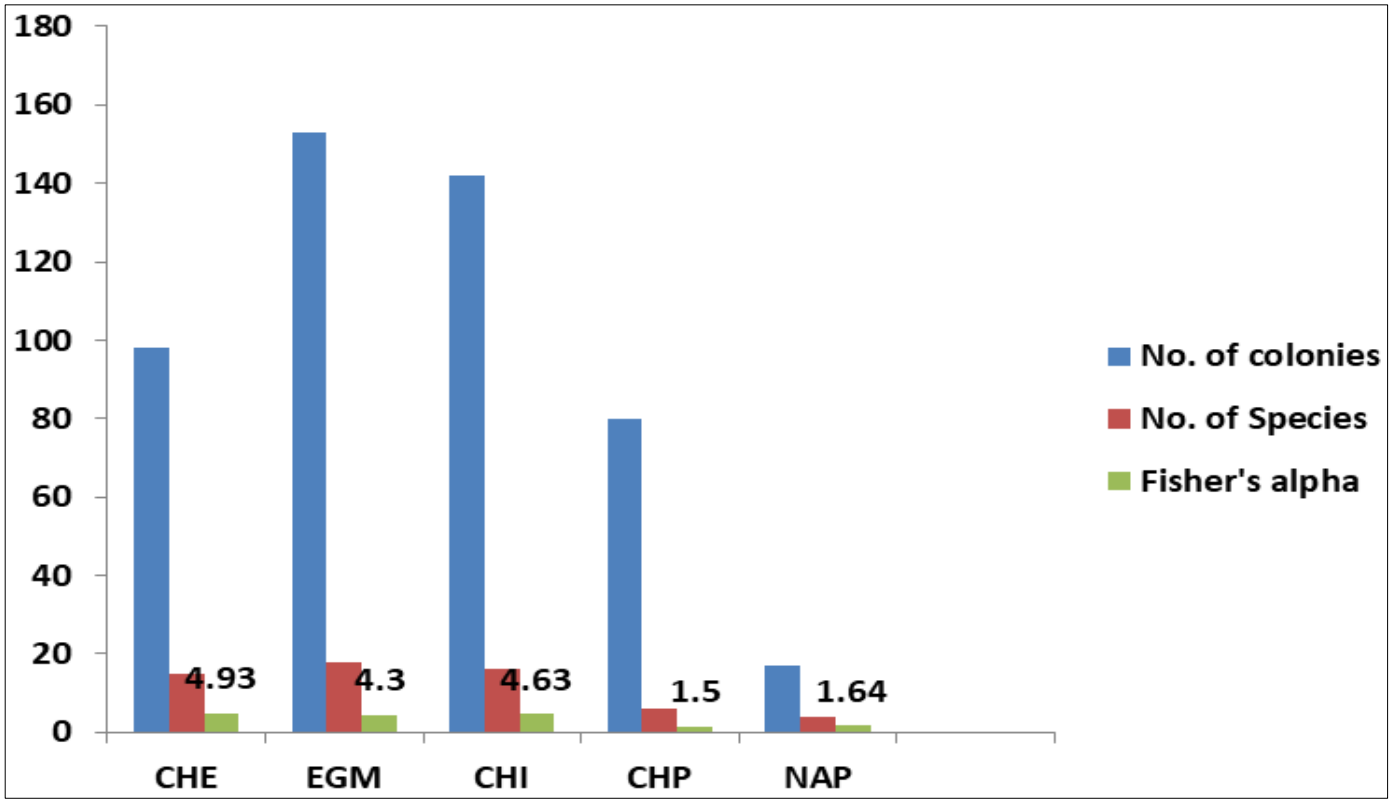
S.NO.	Parameter	Unit	Collection site				
			CHE	EGM	CHI	CHP	NAP
1	Texture		Sandy	Clayey	Clayey	Clayey	sandy
2	Moisture	%	19.6	22.8	26.8	55.6	19.1
3	Nitrogen(as N)	mg/kg	706	1342	2990	1512	1375
4	Phosphorus(as P)	mg/kg	1494.1	1994.9	2905.1	1906.7	3050.5
5	Potassium(as K)	mg/kg	437.2	1002.8	2498.3	1622.8	2071
6	Total Organic matter	%	3	3.9	41.1	26	2.4
7	Total soluble salts	%	1	1	3.5	3.35	3.1
8	pH		6.9	6.5	6.8	6.9	6.3

Che = Chetput, Egm = Egmore, Chi = Chindadripet, Chp = Chepauk, Nap = Napier Bridge

Table 2: Number of Fungal Propagules per gm of soil

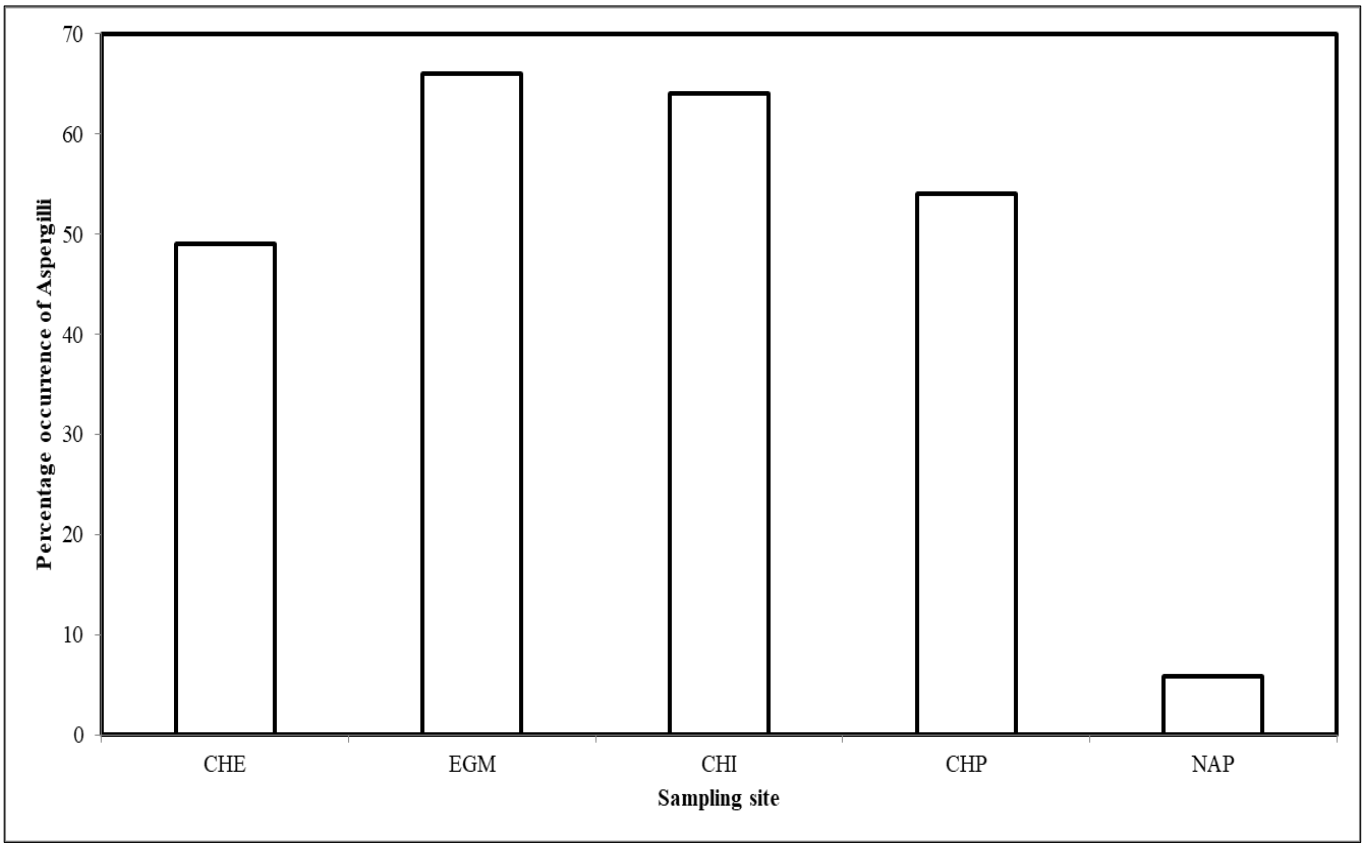
S.No.	Name of fungus	Study Site				
		CHE	EGM	CHI	CHP	NAP
1.	*Aspergillus candidus*			833		
2.	*Aspergillus flavus*	500	333	1333	1166	166
3.	*Aspergillus fumigatus*			333		
4.	*Aspergillus niger*	3500	3833	10,666	4333	
5.	*Aspergillus* sp.1	166	2833			
6.	*Aspergillus* sp.2			1666		
7.	*Aspergillus* sp.3			333		
8.	*Aspergillus terreus*	3166	9833		1666	
9.	*Chaetomium* sp.1			2666		
10.	*Cunnighamella* sp.1	166				
11.	*Curvularia lunata*	1166	333			
12.	*Eurotium* sp.1		1500			
13.	*Gliocladium* sp.1			166		
14.	*Humicola griseus*		166	333		
15.	*Mucor racemosus*	500	833	500		166
16.	*Penicillium* sp.1	3333	833	1000		
17.	*Penicillium* sp.2		333	1000		
18.	*Penicillium* sp.3	166				
19.	*Rhizopus* sp.1	333				
20.	Sterile form CR 1			166	3333	
21.	Sterile form CR 2	166			666	
22.	Sterile form CR 3	333	2000			
23.	Sterile form CR 4		166			
24.	Sterile form CR 5		666			
25.	Sterile form CR 6		500			
26.	Sterile form CR 7		166			
27.	Sterile form CR 8		500			
28.	*Trichoderma* sp.1	166	333	666		
29..	Hyphomycete form CR1			1666		
30.	Yeast form 1	2666		333	2166	2000
31.	Yeast form 2					500

Che = Chetput, Egm = Egmore, Chi = Chindadripet, Chp = Chepauk, Nap = Napier Bridge



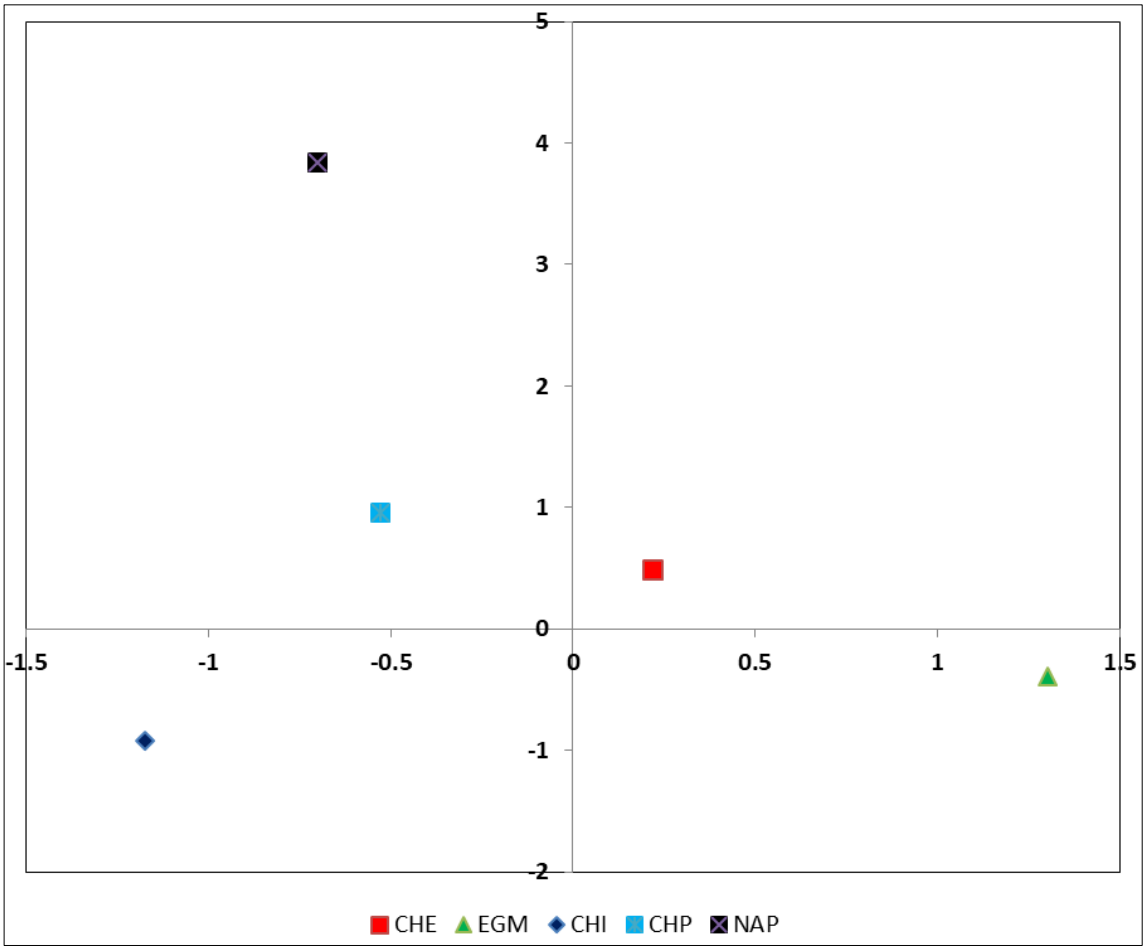
Che = Chetput, Egm = Egmore, Chi = Chindadripet, Chp = Chepauk, Nap = Napier Bridge

Fig 2: Number of colonies, species and Species diversity (Fisher's alpha) of fungi for the Coum River bank soil samples.



Che = Chetput, Egm = Egmore, Chi = Chindadripet, Chp = Chepauk, Nap = Napier Bridge

Fig 3: Percentage Occurrence of Aspergilli in Coum River bank soil



Che = Chetput, Egm = Egmore, Chi = Chindadripet, Chp = Chepauk, Nap = Napier Bridge

Fig 4: Correspondence analysis for the fungal assemblage in Cooum River bank soil

Conclusion

The soil collected from different sites along the Cooum river bank was screened to understand the distribution and diversity of microfungi. A total of 31 fungal species belonging to 22 genera were isolated from the Cooum river bank soil samples of five selected sites. (Table 1). *Aspergillus niger*, *Aspergillus fumigatus*, *Apergillus candidus*, *Aspergillus terreus*, *Eurotium* sp.1, *Curvularia lunata*, *Gliocladium* sp.1, *Humicola griseus* etc., were isolated from the soil samples. On analyzing the number of propagules per gram of soil, *Apergillus niger* recorded highest number in three sites studied, while *Aspergillus terreus* and Yeast form1 dominated in other two sites (Table2). The species diversity was found maximum in the soil samples of the Egmore and found decreasing as it approached the sea in Napier Bridge. (Figure 2). *Aspergillus* represented more number of species. *Aspergillus flavus* could be isolated from all the sites studied (Table 2). The genera *Aspergillus* contributed more than 50 % in the soil samples of three sites studied (Figure 3).

The soil sample collected from Egmore yielded more sterile forms. The correspondence analysis revealed the presence of different microfungal assemblage in the sites studied (Figure 4).

Our study is a preliminary insight into the microfungal distribution and diversity in the soils of Cooum river bank soils. Further studies are required to address and analyse the factors which contribute for the distribution of fungi and their role in this environment.

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